Remarks/Arguments

The Office Action of April 29, 2003 has been carefully considered and the following response prepared. Claims 1-5, 7, 8, 12-14, 24, 27-30, 63, 64, 72 and 73 are canceled without prejudice. Claims 6, 9, 17-20, 23, 25, 26, 60-62 and 65-71 have been amended. Claims 7, 10, 11, 15, 16, 21, 22 and 31-59 are withdrawn from consideration. The specification has been amended to insert section headings and a Brief Description of the Figures.

At page 2 of the Office Action, the Examiner objected to the specification because it contains an embedded hyperlink in paragraph [0111]. Paragraph [0111] has been amended to delete the hyperlink.

At page 2 of the Office Action the Examiner also objected to the specification because it lacks a Brief Description of the Figures and fails to comply with 1.821-825 because sequence identifiers are missing from either the figure legends or the Brief Description of the Drawings.

The specification has been amended to insert a Brief Description of the Drawings that contains reference to the sequence identifiers of the sequences in the drawings.

At page 3 of the Office Action the Examiner objected to informalities in claims 2-6, 9, 13, 17-20, 23-26, 61-71 and 73. Claims 2-5, 13, 24, 26, 63, 64 and 72 have been canceled without prejudice and the objections with respect to these claims are now moot. Claims 6, 17-20, 23, 25 and 26 have been amended to insert an article at the beginning of the claim. Claims 6, 9, 17-20, 23, 25 and 26 have been amended to replace "characterized in that" with "wherein". Claim 19 has been amended to insert "wherein" after "protein" and replace "being" with "is". Claim 18, line 3, has not been amended to insert "wherein the DNA sequence" after "acteyltransferase". The comma between "acetyltransferase" and "under" has been deleted. Applicants submit that claim 18 as amended is not informal. Claim 19 has been amended to insert "the" before "chloroplasts" in line 4. Claims 61, 62, and 65-71 have been amended to insert a comma before "wherein".

At page 4 of the Office Action, the Examiner rejected claims 2-3, 5-6, 13, 17-20, 23-26, 60-61 and 63-72 under 35 USC 112, first paragraph on the basis that the specification is not enabling for method of increasing the production of cysteine, glutathione, methionine and sulfur

derivatives in a plant by transformation with a nucleic acid encoding a cysteine-sensitive SATase or plant SATase rendered cysteine-insensitive by mutagenesis.

Applicants traverse this rejection. Claims 2-3, 5, 13, 24, 63, 64 and 72 have been canceled without prejudice. Independent claim 60 has been amended to state that the nucleic acid sequence encodes a cysteine-insensitive serine acetyltransferase which the Examiner indicated is enabled. Claims 6, 17-20, 24, 63 and 64 depend directly or indirectly from claim 60 and are also amended by the amendment to claim 60. New claims 74 and 76 also state that the nucleic acid sequence encodes a cysteine-insensitive serine acetyltransferase. Withdrawal of this section 112, first paragraph rejection is requested.

At page 8 of the Office Action, the Examiner rejected claims 4-5 and 60 under 35 USC 112, first paragraph as failing to contain a written description of the invention because the specification does not disclose a nucleic acid sequence encoding an SATase that has been converted from a cysteine-sensitive SATase to a cysteine-insensitive one.

Applicants traverse this rejection. Claims 4 and 5 have been canceled without prejudice. Claim 60 has been amended to state that the nucleic acid sequence encodes a cysteine-insensitive serine acetyltransferase. Withdrawal of this section 112, first paragraph rejection is requested.

At page 9 of the Office Action, the Examiner rejected claims 2-6, 9, 13, 17-20, 23-26 and 60-73 under 35 USC 112, second paragraph as being indefinite. The Examiner objected to the term "increasing" in claims 60 and 72, stating that "increasing" is a relative term and suggested that claims 60 and 72 be amended to state that the levels are compared to the levels in a non-transformed plant. Claim 60 has been amended as suggested by the Examiner to state that the increase is "in comparison with the level observed in nontransformed plant cells". Claim 72 has been canceled and rewritten as new claims 74 and 76 that also contain the foregoing limitation.

The Examiner also objected to the location of the phrase "or in plants containing plant cells" in claim 60. Claim 60 has been amended as suggested by the Examiner.

The Examiner also objected to the use of "overexpressing" and "expressing" in claims 60 and 70-73, stating that it is not clear what the practitioner must do to overepxress or express the serine acetyltransferase in plants or plant cells.

Applicants submit that the terms "overexpressing" and "expressing" are not indefinite and that persons skilled in the art understand what it means to overexpress or express a protein.

Overexpress refers to production of a protein by cells or organisms transformed with a nucleic acid sequence encoding the protein, where the cells or organisms also endogenously produce the protein or its analogue. Expressing refers to the production of a protein by cells or organisms transformed with a nucleic acid sequence encoding the protein.

In view of the above, withdrawal of this entire section 112, second paragraph rejection is requested.

At page 10 of the Office Action, The Examiner rejected claims 72 and 73 under 35 USC 112, second paragraph as being incomplete for omitting essential steps involved in production of plants.

Applicants traverse this rejection. Claims 72 and 73 have been canceled and rewritten as new claims 74-77. Claim 76 is directed to a method of increasing the production of cysteine, glutathione, methionine or sulfur-containing derivatives of methionine by plants that contains a step of regenerating plants from the transformed plant cells. Withdrawal of this 35 USC 112, second paragraph rejection is requested.

At page 11 of the Office Action, the Examiner rejected claims 2-6, 13, 17, 19-20, 24, 26, 60-63, 65 and 72-73 under 35 USC 103(a) as being unpatentable over Saito *et al.* (1994) Plant Physiol. 106: 887-895 in view of each of Noji *et al.* (1998) J. Biol. Chem. 273: 32739-32745) and Ruffet *et al.* (1995) Eur. J. Biochem. 227: 500-509. The basis for this rejection is that it would have been obvious for one skilled in the art to modify the method of increasing the production of cysteine in a plant by overexpressing a cytoplasmic cysteine synthase in the cytoplasm and chloroplasts of a plant as taught by Saito *et al.* and to use a nucleic acid encoding another enzyme required for cysteine biosynthesis, SATase, as described by each of Noji *et al.* and Ruffet *et al.*. The Examiner further stated that one skilled in the art would have been motivated to do so because of the role SATase has in regulation of cysteine biosynthesis (Noji *et al.* page 32744, left column, paragraph 4) and because Saito *et al.* suggests expressing SATase in the plants for maximal cysteine formation (page 893, left column, paragraph 1). The Examiner additionally stated that because of the open claim language in the claimed method, expression of both cysteine synthase and SATase is not excluded.

Applicants traverse this rejection.

Saito *et al.* discloses overexpression of cysteine synthase in tobacco plants transformed to express spinach cytoplasmic cysteine synthase. The Examiner again cited page 893, left column, first paragraph as suggesting overexpression of SAT. This paragraph of Saito *et al.* discusses a working model for the regulatory mechanism of cysteine biosynthesis in chloroplasts accumulating overexpressed cysteine synthase. As discussed in Applicants' response to the previous Office Action, there is no suggestion in this paragraph for overexpressing SAT. The only possibly relevant sentence states: "Overexpressed CSase in the transgenic plants may require more Ser acetyltransferase, leading to enhanced availability of OAS for maximal Cys formation." This sentence falls far short of suggesting overexpression of SAT, and, even if it could be construed to suggest overexpression of SAT, can only mean that both cysteine synthase and SAT must be overexpressed for maximal cysteine formation.

In the present Office Action, the Examiner pointed out Figure 6 and page 891 left column, paragraph 2 to page 892, left column paragraph 2 as showing that transformed plants have much increased levels of cysteine over non-transformed plants.

This portion of Saito *et al* does not support the Examiner's assertion of increased levels of cysteine in plants transformed with cysteine synthase. Page 891 left column, paragraph 2 to page 892, left column paragraph 2 and Figure 6 form the section of Saito *et al.* entitled "Cys Production in Isolated Chroloplasts Responding to Addition of Biosynthetic Precursors". This section of Saito *et al.* discusses experiments on modulation of cysteine biosynthesis in isolated chloroplasts of transformed 4F plants expressing cysteine synthase and nontransformed control plants in response to addition of the biosynthetic precursors sulfide, sulfite, sulfate and OAS (O-acetylserine). Figure 6 shows cysteine levels in the isolated chloroplasts of the transformed 4F15 and control SR1 plants upon the addition of biosynthetic precursors alone or in various combinations. As can be seen in Figure 6, when no precursors were added the cysteine levels of the chloroplasts from control and transformed plants were the about the same. Addition of sulfide, sulfite or sulfate alone did not increase cysteine production. However, the addition of OAS alone increased cysteine production and the addition of combined OAS and sulfur compounds resulted in more increased cysteine prodution. This section of Saito *et al.* thus shows that the only increase in cysteine levels in plants transformed with cysteine synthase over those found in nontransformed chloroplasts were obtained by adding biosynthetic precursors from an

external source. No increase of cysteine levels related to the overexpression of cysteine synthase in the absence of externally supplied biosynthetic precursors was found.

Noji *et al.* discloses experiments on the subcellular localization of SAT and feedback regulation of the three SAT forms from *A. thaliana*. Experiments were performed using *E. coli* or plants transformed with fusion proteins comprised of an SAT N-terminal fragment fused to jellyfish green fluorescent protein (GFP). Noji *et al.* discusses the putative role of SAT in regulation of cysteine biosynthesis by analogy to bacterial SAT. There is no suggestion in Noji *et al.* that overexpression of SAT in plant cells would increase production of cysteine.

Ruffett *et al.* discloses experiments on the subcellular distribution of SAT from *Pisum sativum* and isolation and characterization of a cytosolic isoform of SAT from *A. thaliana* referred to as SAT5 . There is no suggestion in Ruffett *et al.* that cysteine production in plants or plant cells could be increased by overexpressing SAT.

Claims 2-5, 13, 24, 63, 72 and 73 have been canceled without prejudice and this rejection is now moot with respect to those claims. Claims 72 and 73 have been rewritten as new claims 74-77. Claim 60 as presently amended and new claims 74 and 76 which replace canceled claim 72 state that the method "consists of" the recited steps. Claims 6, 17, 19, 20, 26, 61 and 65 depend directly or indirectly from claim 60 and are also amended by the amendment to claim 60.

It is well-established that before a conclusion of obviousness may be made based on a combination of references, there must be a reason, suggestion or motivation in the prior art to lead an inventor to combine those references. Saito *et al.* discloses experiments using a <u>different</u> enzyme, cysteine synthase. At best, Saito *et al.* arguably suggests overexpression of both cysteine synthase and SAT. Noji *et al.* and Ruffet *et al.* do not cure the deficiencies of Saito *et al.* Noji *et al.* discloses experiments of feedback regulation and subcellular localization of SAT, but does not suggest that overexpression of SAT in plant cells could increase production of cysteine. Ruffet *et al.* discloses experiments on the subcellular distribution of SAT from *Pisum sativum* and isolation and characterization of a cytosolic isoform of SAT from *A. thaliana* referred to as SAT5, but there is no suggestion in Ruffett *et al.* that cysteine production in plants could be increased by overexpressing SAT. There is thus no suggestion, reason or motivation in the cited prior art to overexpress SAT alone to increase production of cysteine, glutathione, methionine or sulfur-containing derivatives of methionine by plant cells and plants.

Even assuming *arguendo* that the combination of references is proper, the combined disclosures of the references are insufficient to support the Examiner's conclusion that the claimed methods of increasing the production of cysteine is obvious. The combined disclosures of Saito *et al.*, Noji *et al.* and Ruffet *et al.* still fail to suggest that cysteine production could be increased by overexpressing SAT alone in plant cells or plants as claimed. The combined disclosures of Saito *et al.*, Noji *et al.* and Ruffet *et al.* at best suggest that overexpression of both cysteine synthase and SAT are necessary to obtain increased production of cysteine and glutathione, even if such an increase would be possible. Applicants' claimed methods are not obvious over Saito *et al.* in view of Noji *et al.* and Ruffet *et al.*. Withdrawal of this section 103 rejection is requested.

At page 13 of the Office Action, the Examiner rejected claim 18 under 35 USC 103(a) as being unpatentable over Saito *et al.* Plant Physiol. 106: 887-895, 1994 in view of each of Noji *et al.* (1998) J. Biol. Chem. 273: 32739-32745) and Ruffet *et al.* (1995) Eur. J. Biochem. 227: 500-509 as applied in the immediately preceding rejection, and further in view of Svab *et al.* (1993) Proc. Nat'l Acad. Sci. USA 90: 913-917. The basis for this rejection is that it would have been obvious to one of ordinary skill in the art to increase the production of cysteine and other sulfur-containing compounds in a plant or plant cells by transformation with a construct encoding a cytosolic SATase fused to a chloroplast transit peptide sequence as taught by Saito *et al.* in view of each of Noji *et al.* and Ruffet *et al.* and to modify that construct to transform the chloroplast as described in Svab *et al.* The Examiner argued that one skilled in the art would be motivated to do so because the introduction of protein into the choloroplast by transformation or nuclear transformation with a construct that has a chloroplast transit peptide is an obvious design choice.

Applicants traverse this rejection. Svab *et al.* discloses increased frequency of plastid transformation using a plasmid containing a tobacco *Sac*II-*Eco*RV plastid fragment wherein a chimeric gene is inserted between the rbcL gene and open reading frame ORF512. Svab *et al.* has nothing in common with the present invention beyond the disclosure of a method for transforming chloroplasts that could be used transform chloroplasts with SAT.

The addition of Svab *et al.* does not cure the deficiencies of Saito *et al.*, Noji *et al.* and Ruffet *et al.* The combined disclosures of Saito *et al.*, Noji *et al.*, Ruffet *et al.* and Svab *et al.* still fail to disclose increasing the production of cysteine in plants by overexpressing SAT alone

as claimed, or any suggestion that doing so would increase production of cysteine. Claim 18 is therefore not obvious over Saito *et al.* in view of Noji *et al.* Ruffet *et al.* and Svab *et al.* Withdrawal of this section 103(a) rejection is requested.

At page 14 of the Office Action, the Examiner rejected claims 23, 25, and 66-71 under 35 USC 103(a) as being unpatentable over Saito *et al.* Plant Physiol. 106: 887-895, 1994 in view of each of Noji *et al.* (1998) J. Biol. Chem. 273: 32739-32745) and Ruffet *et al.* (1995) Eur. J. Biochem. 227: 500-509 as applied in the preceding rejections, and further in view of LeBrun *et al.* (1999) RE 36,449. The basis for this rejection is that the combined teachings of Saito *et al.*, Noji *et al.* and Ruffet *et al.* disclose plants transformed with a construct encoding SATases fused to a RuBisCO ssu chloroplast peptide sequence or their native transit peptide and that it would have been obvious to substitute the optimized transit peptide taught by LeBrun *et al.* as an obvious design choice.

Applicants traverse this rejection. LeBrun *et al.* RE 36,449 discloses an optimized transit peptide that comprises a first chloroplast transit peptide from a ribulose-1,5-biphosphate carboxylate small subunit, an N-terminal domain of a mature ribulose-1,5-bisphosphate carboxylate small and subunit protein and a second chloroplast transit peptide from a ribulose-1,5-bisphosphate carboxylate small subunit. There is no suggestion in LeBrun *et al.* that cysteine production in plants or plant cells could be increased by overexpressing SAT.

The addition of LeBrun *et al.* does not cure the deficiencies of Saito *et al.*, Noji *et al.* and Ruffet *et al.* The combined disclosures of Saito *et al.*, Noji *et al.*, Ruffet *et al.* and LeBrun *et al.* still fail to disclose increasing the production of cysteine in plants by overexpressing SAT alone as claimed, or any suggestion that doing so would increase production of cysteine. Claim 14 is therefore not obvious over Saito *et al.* in view of Noji *et al.*, Ruffet *et al.* and LeBrun *et al.* Withdrawal of this section 103(a) rejection is requested.

In view of the above, the present application is believed to be in a condition ready for

allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

Respectfully submitted,
CONNOLLY BOVE LODGE & HUTZ LLP

Date: August 29, 2003

By: Liza D. Hohenschutz

Liza D. Hohenschutz
Reg. No. 33,712
P.O. Box 2207
Wilmington, Delaware 19899
(302) 888-6420
Attorney for Applicants

285208